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Impact of Sulfur Compounds on the Activity of Bacterial L-methioninase

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Abstract:

The enzyme L-methioninase (EC 4.4.1.11) is present in all microbial species, while completely absent from mammals. This enzyme mainly catalyzes the α,γ-elimination of L-methionine, resulting in the production of α-ketobutyrate, methanethiol, and ammonia. The objective of this work was to achieve partly purification of L-methioninase from *Staphylococcus sciuri*. The enzyme was isolated and partially purified by the use of 80% ammonium sulfate precipitation. A range of concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mM) of four thiol compounds, namely cysteine, N-acetyl cysteine, thioglycolate, and glutathione, were examined in the reaction mixture to assess their impact on L-methioninase. Although all the substances examined exhibited enzyme activation at lower concentrations, higher concentrations functioned as inhibitors. Given that this enzyme is therapeutic, the results indicate that including the tested chemicals with its lower concentrations into the purified enzyme will improve its activity.

Key words: Enzymes, L-methioninase, *Staphylococcus sciuri*, Sulfur Compounds.

1. Introduction

L-methioninase, alternatively referred to as methionine-gamma lyase, L-methionine lyase, and L-methionine demethylase, is an enzyme typically classified as a member of the aspartate aminotransferase family of pyridoxal-5-phosphate (PLP) dependent enzymes. In bacterial species, L-methioninase is commonly found as an internal enzyme, when in fungus it is an external enzyme. Its medicinal utilisation is restricted to microbial and fungal sources due to

its conspicuous absence in mammals **(Suganya *et al.*, 2017).** L-methioninase is a microbial enzyme that has significant therapeutic potential given its reported potency as an anticancer agent against several tumor cell lines including breast, lung, colon, kidney, and glioblastoma **(Sharm *et al.*, 2014).** The presence of this enzyme has been detected in distinct microorganisms, such as yeast **(Monnet *et al.*, 2013)** and bacteria **(Kannan and Marudhamuthu, 2019).** Furthermore, plants encode L-methioninase **(Reda *et al.*, 2023).** Significantly, this enzyme is not present in mammalian cells **(Swathi and Sridevi, 2015).** The large-scale synthesis of L–methioninase from microbial sources is facilitated by their straightforward production techniques. The limitation of L-methionine is a fundamental strategy in the metabolic regulation of malignancies and relies on methionine for both survival and growth. Constrained intake of L-methionine results in significant increases in longevity in fruit flies **(El-Shora *et al.*, 2021).** Due to the remarkable performance of microbial enzymes, which function effectively under a wide range of physical and chemical circumstances, enzyme manufacturing is essential in several industrial areas. Notably, L-methioninase is among the limited number of enzymes that have substantial therapeutic promise **(Selim *et al.*, 2015).** Therefore, our work specifically concentrated on L-methioninase derived from bacteria for uses in therapy. The objective of this study was to examine the impact of sulfur compounds on the enzymatic activity of L-methioninase derived from *Staphylococcus sciuri.*

2. Materials and Methods

Materials

All of the other chemicals were of analytical grade and purchased from Sigma.

Microorganism media and growth conditions

The *Staphylococcus sciuri* strain utilized in this work was obtained from the Clinical Microbiology Lab at the Faculty of Medicine, Mansoura University, located in the Dakahlia Governorate of Egypt. To conduct solid-state fermentation (SSF) of *Staphylococcus sciuri*, 10 grammes of soybean meal with a particle size ranging from 0.4 to 0.8 centimetres is used as the substrate. This meal is moistened with 10 millilitres of a 0.01 M phosphate buffer at a pH of 7.4, together with 5 grammes of L-methionine. This mixture is transferred into 250 ml Erlenmeyer flasks and sterilized by subjecting it to autoclaving at 121°C for 15 minutes at a pressure of 15 lb/inch. A 3 ml volume of the bacterial suspension was added to the flasks and then incubated at 37°C for 4 days under static conditions. At the conclusion of the fermentation phase, 90 ml of a 0.01 M phosphate buffer with a pH of 7 was introduced into the cultured medium. The solution was agitated for 15 minutes, followed by centrifugation at 8,000 revolutions per minute for 20 minutes. A crude

enzyme preparation was made from the cell-free supernatant obtained.

Extraction of L-methioninase

After the appropriate fermentation period, a phosphate buffer containing 0.01 M of phosphate and a pH of 7 was added to the cultured medium to separate the extracellular crude enzyme. The mixture underwent 15 minutes of agitation followed by 20 minutes of centrifugation at 8,000 revolutions per minute. Collected cell-free supernatant was utilized as the crude enzyme preparation following the method described by **(El-Shora *et al.*, 2011).**

Partial purification of L-methioninase

Enzyme stability and activity were ensured by purifying the crude extract at 4°C. To reach 80% saturation, ammonium sulfate was added to the mixture, which was then left at 4°C for 12 hours. The precipitate was collected by centrifuging the solution at 8,000 revolutions per minute for 20 minutes at 4°C. The precipitate was then dissolved in a 0.01 M phosphate buffer with a pH of 8.5 and the mixture was dialyzed overnight at 4°C against the same buffer. This process yielded the dialyzed ammonium sulfate fraction. Enzyme activity was measured in this fraction in relation to the protein abundance.

Assay of L-methioninase and protein determination

Assay of L-methioninase was conducted following the method described by (**El-Shora *et al.*, 2021).** The protein concentration was measured with the Bradford (1976) method.

Effect of sulfur compounds on L-methioninase activity

Study was conducted to examine the impact of cysteine, N-acetyl cysteine, thioglycolate, and glutathione on the activity of L-methioninase at different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mM) in the reaction mixture. Additional control samples, devoid of any of these chemicals, were also prepared. Specified enzyme activity was quantified in units and reported as a proportion relative to the control.

3. Results

The enzyme was derived from *Staphylococcus sciuri* and contained 80% ammonium sulfate. Its specific activity was measured to be 15.0 units mg-1 protein equivalent. The present study examined the impact of four thiol compounds on the action of partly pure L-methioninanse. The chemicals in question were hydrogenated cysteine, N-acetyl cysteine, thioglycolate, thiothreitol, and glutathione. Each compound was evaluated at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mM in the reaction medium. Isolated control samples devoid of these chemicals were prepared. The data in Figure 1 demonstrate that the enzyme activity is increased in the presence of cyateine. The ideal concentration was found to be 0.6 mM, and the figure exhibited a bell-shaped distribution.

According to Figure 2, N-acetyl cysteine activated L-methioninase at an ideal dose of 0.6 mM, with a significant decrease observed at concentrations of 0.8 and 1 mM. The data shown in Figure 3 illustrate that the activity is increased by thioglycolate at lower concentrations up to 0.6 mM, but thereafter decreases at greater concentrations. The data shown in Figure 4 demonstrate that glutathione, when present as a tripeptide, stimulated enzyme activity up to a concentration of 0.4 mM. Subsequently, it suppressed the activity at concentrations of 0.6, 0.8, and 1 mM.

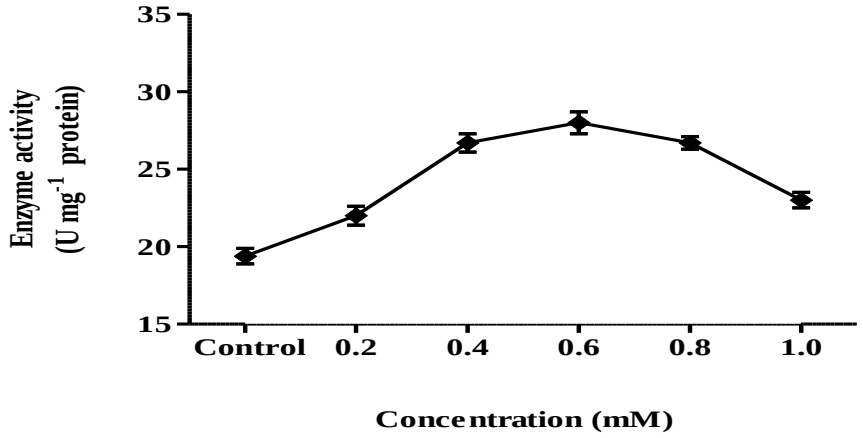

Fig. 1: Influence of cysteine on L-methionine activity.

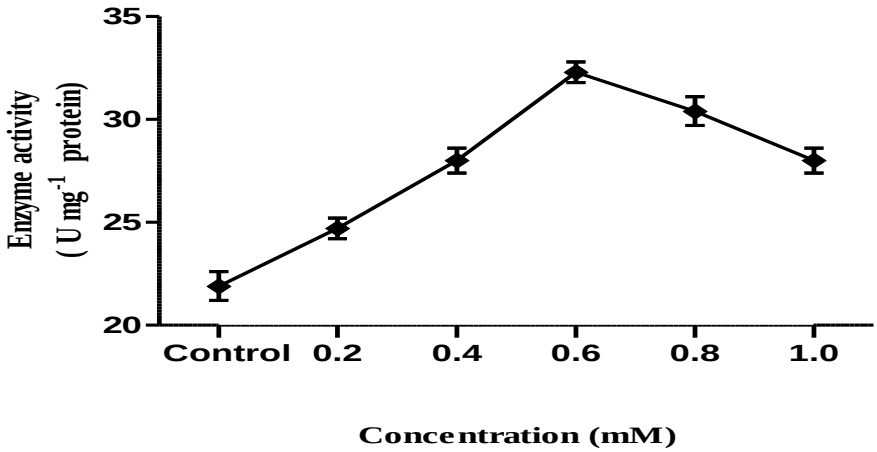

Fig. 2: Influence of N-acetyl cysteine on L-methionine activity.

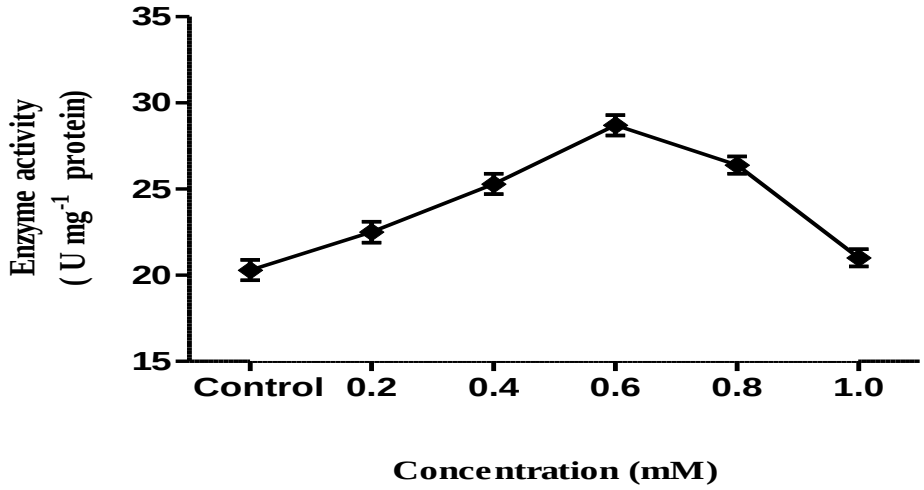

Fig. 3: Influence of thioglycolate on L-methionine activity.

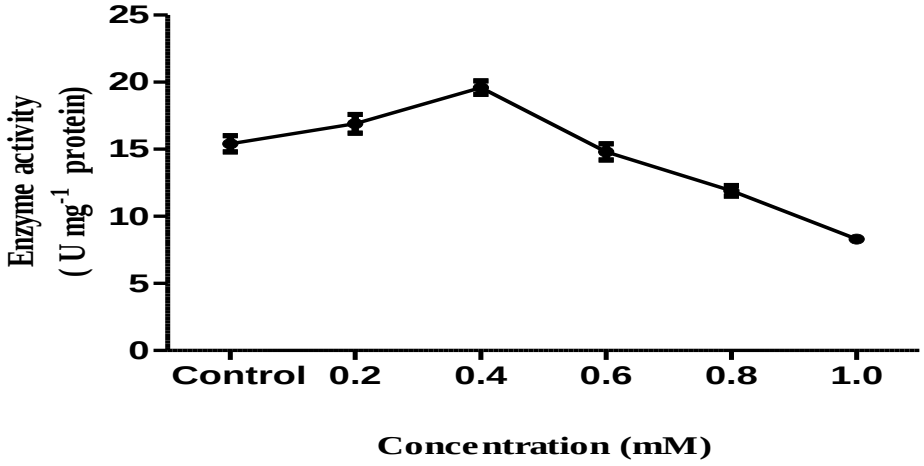

Fig. 4: Influence of glutathione on L-methionine activity.

4. Discussion

Enzymes are specialized biocatalysts that exhibit a high degree of specificity towards the reactions they facilitate. These complex macromolecules expedite the metabolic processes of the body and carry out specialized functions with exceptional effectiveness. Enzymes have the ability to accelerate chemical processes by up to one million times compared to alternative catalytic approaches **(Blanco & Blanco, 2017).** Purification is the mechanical separation of an enzyme from other constituents in the extraction media, where it may be present as a minute proportion of the overall protein. Hence, it is crucial to construct the early phases of enzyme

purification in a manner that maximizes the removal of undesired proteins, employing the most straightforward and efficient techniques now accessible **(Chapman *et al.,* 2018; El-Shora *et al.,* 2018).** Cysteine, N-acetyl cysteine, thioglycolate, and glutathione (GSH) bind to L-methioninase and function as activators when added to the reaction medium. Cysteine, an amino acid, has been shown to specifically stimulate the activity of acid phosphatase and asparaginase enzymes **(El-Shora and Metwally, 2009; Warangkar and Khobragade, 2010).** In their study, (**El-Shora *et al.,* 2024)** documented the augmentation of L-arginine deiminase activity in *Penicillium chrysogenum* by the exposure to thiol compounds, including cysteine and thiothreitol. The study conducted by (**El-Shora *et al.,* 2011)** revealed that dithiothreitol, cysteine, and glutathione (GSH) enhanced the activity of various bacterial enzymes, including cholesterol oxidase. Among thiol compounds, dithiothreitol has been shown to activate fungal β-glucanase by shielding the enzyme's sulfhydryl groups from oxidation **(El-Shora *et al.,* 2019).** Additionally, cysteine has been shown to boost the activity of other microbial enzymes such as asparaginase **(Warangkar *et al.,* 2010)** and laccase **(El-Shora *et al.,* 2021).** Enzyme activation by thiol compounds, such cysteine and other thiol-containing molecules, suggests that these chemicals shield the enzyme's sulfhydryl groups through the reaction. The maintenance of enzyme stability and activity may be vitally dependent on this protection. Moreover, these thiol compounds have the potential to lower the Km value of the enzyme, therefore reducing its binding affinity to the substrate and increasing enzyme activity, especially at lower concentrations **(El-Shora *et al.,* 2024).** Finally, L-methioninase is a cytosolic inducible enzyme that is produced by the addition of L-methionine to the culture medium. This study emphasizes the significance of thiol compounds in stimulating the synthesis of L-methioninase from *Staphylococcus sciuri.* Strategically limiting methionine could be an effective method for regulating cancer development, especially in malignant tumors that depend on methionine for survival and proliferation. Furthermore, the inclusion of L-methionine in the human diet is necessary to promote the production of L-cysteine.

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الملخص العربى

تأثير مركبات الكبريت علي نشاط إنزيم الميثيونينيز البكتيري

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يتواجد إنزيم الميثيونينيز (EC 4.4.1.11) في جميع الكائنات الحية باستثناء الثدييات. إنه يحفز في المقام الأول تكسير الحامض الأميني الميثيونين لإنتاج ألفا كيتوبيوتريت، و ميثانثيول، والأمونيا. كان الهدف من هذه الدراسة هو تنقية الإنزيم جزئيًا من بكتريا استافيلوكوكس سكيوري. تم عزل الإنزيم وتنقيته جزئيا باستخدام كبريتات الأمونيوم (80%). تم اختبار تأثيرات 4 مركبات كبريتية وهي السيستين (حامض أميني)، N-أسيتيل سيستين، ثيوجليكولات، والجلوتاثيون على نشاط إنزيم الميثيونينيز بتركيزات مختلفة (0.2، 0.4، 0.6، 0.8، و 1.0 مللي مولار) وذلك في وسط التفاعل. جميع المركبات التي تم اختبارها قامت بتنشيط الإنزيم عند التركيزات الأقل. بينما التركيزات المرتفعة كانت بمثابة مثبطات للنشاط الإنزيمي. وتشير النتائج إلى أن إضافة المركبات المختبرة بتركيزات منخفضة إلى الإنزيم المنقى جزئيا يحفز نشاطه و تتمثل أهميته في أنه إنزيم علاجي للسرطان.